

oligonucleotides were mixed, at the same concentration (1: g: l), with 5: l of 10 X Klenow buffer (500mM Tris, pH7.2, 100mM MgSO₄, 1mM dithiothreitol) and 18: l distilled water. The reaction mixture was heated and maintained at 80°C for 5 min and then allowed to cool to 37°C, followed by addition of 5 :l of 2.5mM deoxyribonucleotides (dNTP) and 2 :l of Klenow fragment (Promega) to initiate DNA synthesis at room temperature. After 40 minutes EDTA was added to a final concentration of 10 mM to stop the reaction, and this was followed by incubation at 75°C for 5 min to inactivate the Klenow enzyme. The double-stranded DNA segments were purified by phenol/chloroform extraction and ethanol precipitation, and dissolved in 50 :l of distilled water. To assemble the three segments into a longer DNA fragment, half of double-stranded segments 1, 2 and 3 were double digested with BamHI-KpnI, KpnI-Sau3A1 and Sau3A1-EcoRI, respectively. The digested segments were recovered by gel purification, using low-melting-temperature agarose, and dissolved in 25 :l of water. About 0.1 :g of digested segment 1 was ligated into 0.1 :g of pBluescript vector (stratagene) that had been predigested with BamHI and KpnI. The ligation was performed overnight at 4°C in a 20 :l reaction mixture. Similarly, 0.2 :g each of segment 2 and segment 3 were ligated together into pBluescript vector (Stratagene) that had been predigested with KpnI and EcoRI. 10 :l of each ligation reaction mixture was transferred into 100 :l of *E. coli* competent cell (XL1-Blue strain, Startagene) according to the standard protocol described by Sambrook, J., Fritsch E.F. and Maniatis, T. ("MOLECULAR CLONING, A LABORATORY MANUAL" 2nd edition, Cold Spring Harbor Laboratory Press, New York, 1989). The resulted plasmids were isolated with Qiagen Plasmid Isolation Kit (Qiagen) and sequenced from both ends for confirmation. The verified segment 1 and segments 2 and 3 were removed from pBluescript by digestion with BamHI-KpnI and KpnI-EcoRI respectively. 0.1 :g of verified segment 1 and segments 2 and 3 each were ligated into